Identification of Bacterial Strains Isolated from Patients with Urinary Tract Infection and the Role of Plasmids in their Antibiotic Resistance

Zeena Gh. Faisal
Falah A. J. Attawi
Ghalib H. Al-Bakiri
Dept. of Biotechnology/College of Science/ University of Al -Nahrain

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Abstract

One hundred fifty bacterial strains were isolated from patients with urinary tract infections (UTIs). They were belong to ten different species of gram-negative bacteria and to two genera of gram–positive bacteria. *E. coli* was the major causative agent and comprise 40% of all cases. *Klebsiella pneumoniae* and *Proteus mirabilis* were second and third with 18.67% & 18.0% respectively. Other gram-negative bacteria were belong to the genera *Enterobacter, Acinitobacter, Pseudomonas, Citrobacter* and *Serratia*. Ten cases (6.67%) were caused by genus *Staphylococcus* and seven (4.66%) were caused by *Streptococcus*. Out of the 150 positive cases, 96(64%) were from female patients, while 54(36%) were from males.

High percentage of all isolates were resistant to all used antibiotics except for the nitrofuranation to which all isolates were sensitive . the second most effective antibiotic for all isolates was Nalidixic acid (25% of all isolates were resistant), while the Gentamycin was the third most effective antibiotic (39% were resistant). 75% of *E. coli* isolates and 86% of all isolates were resistant to Ampicillin. High percentages of resistance to antibiotics is a reflection for the misuse of antibiotics.

Conjugation experiments showed that *E. coli* strains harbor a self transmissible plasmid carrying resistant genes for tetracycline (TE^r), Chloramphenicol (C^r), streptomycin (S^r) and Trimethoprime (SXT^r). It seems that this plasmid is widely spread in the Iraqi isolates since it was detected in all the three examined *E. coli* strains. No conjugation was detected between *E. coli* and other members of *Enterobacteriaceae* like *proteus*, *Serratia* and *Acinitobacter*.

Keywords: Bacteria, Resistance to Antibiotics, plasmids

Introduction

The urinary tract is one of the most common sites of bacterial infection, particularly in females; 10-20% of the women have a urinary tract infection (UTI) at some time in their life and a significant number have recurrent infection [1]. UTI is a disease of worldwide importance and in some instances it threatens renal function and may be life [2]. However a considerable proportion of the population may acquire asymptomatic infection [5]. It may involve just the lower tract or both lower and upper tracts [6].

Urinary tract infections are caused by gram-positive and gram-negative bacteria. However the vast majority of infections are caused by *Enterobacteriaceas* originating from the gut [7]. The most common bacteria causing UTIs is *Escherichia coli* [2,3,4,8,9]. *Proteus mirabilis* is often associated with urinary stone. *Klebsiella, Enterobacter, Serratia* and *Pseudomonas aeuroginosa* are more frequently found in hospital acquired UTI [1]. Gram- positive cocci cause UTIs less often than gram-negative bacteria [10]. Distribution of bacteria causing UTIs and their antibiotic sensitivities vary from place to place and from time to time depending on the environment and the choice of treatment which require a continuous assessment to establish the resistance pattern of the microorganisms [11,12,13,14,15].

The wide spread of bacterial strains resisting several antibiotics became one of the major problems in treating the UTI. The antibiotic resistance can be coded by chromosomal or plasmid genes. Many of the plasmids carrying antibiotic resistance genes can be transferred from one bacterial cell into another by conjugation, thus spreading the resistance to antibiotics [16]. The association of transmissible plasmids with the multiresistance to several antibiotics were established in several bacterial spp. causing UTIs [17,18].

In this study the distribution and antibiotic sensitivity of bacterial strains isolated from UTIs were determined, and the role of transmissible plasmid in the resistance to some antibiotics was investigated.

Materials and Methods

Sample collection and culture: Midstream urine samples were collected in sterile tubes from patients in Medical City Hospital and Al-Hilla Hospital during the period from 20.6.1998 to 10.8.1998. Loopful of undiluted urine samples were spread on blood agar plates (Oxoid) and MacConkey's agar plates (Difco), and inclubated overnight at 37°C.

Bacterial identification: Gram-negative bacterial isolates were identified by the API 20E system (API Bio Merieux, Lyon, France) that designed for the performance of 20 standard biochemical tests. Gram-positive isolates were identified by microscopic examination and some biochemical tests.

Antibiotics susceptibility testing: Susceptibility to antibiotics were determined for all bacterial isolates by standard disk diffusion method [19], using 12 commercially available disks (Al-Raze Center Disks). The following antibiotics were tested: Ampicillin (AM, 25µg), Tetracycline (TE, 30µg), Rifampicin (RA, 5µg), Cephalexin (CX, 30µg), Streptomycin (S, 10µg), Chloromaphenicol (C, 30µg), Neomycin (N, 30µg), Gentamycin (GM, 10µg), Amoxicillin (AMX, 10 µg), Nalidixic Acid (NA, 30 µg), Nitrofurantion (FT, 300µg), and Trimethoprime + Sulfamethoxazole (SXT, 1.25µg - 23.75µg).

Minimum inhibitory concentrations (MICs) were determined on nutrient agar plates (Oxoid) by agar dilution method [20].

Bacterial Conjugation: Conjugation between donor and recipient *E. coli* HB 101 strain was performed on solid surface as described by [21]. Cells of donor and recipient strains were grown in Brain Heart Infusion Broth (BHIB) to mid log phase (O.D = 0.35). 0.5ml of donor and recipient cultures were mixed and filtered through a millipore filter (0.45um). The filter was laid on the surface of nutrient agar plate and incubated at 37°C for 6 hours. Cells were

washed of the filter paper with fresh BHIB, diluted and plated on nutrient agar plates containing the appropriate antibiotics to select for the transconjugants. Controls of both donor and recipient cells were treated exactly in the same manner as above to check background spontaneous mutation frequencies. Conjugation frequency was expressed as the number of transconjugants per donor cells in the mating mixture.

Results and Discussion

Isolation and Identification of bacterial strains: Suspected bacterial colonies were picked up from blood agar and MacConkey's plates and identified by microscopic examination and biochemical tests. The gram-negative rods were identified by using the API 20E system, while the gram-positive cocci were identified by microscopic examination and catalase and coagulase tests.

One hundred and fifty urine samples were positive (i.e. more than 10^5 Bacteria / ml were detected). Microscopic and biochemical identification showed that the causative agents were belong to ten different species of gram–negative rod bacteria and to two genera of gram-positive bacteria (Table-1). *E. coli* was the major causative agent and comprise 40% of all cases (Table-1). This is in agreement with what is known about the *E. coli* as the major cause of UTI worldwide [1,2,10,22]. All other gram-negative bacterial spices isolated during the study are common cause of UTIs worldwide. However their prevalence are different from one place into another [1,2,10]. The frequency of infection with *K. pneumoniae* and *P. mirabilis* have approximately the same rate compared with previous studies that showed that *P. mirabilis* was the second causative agent of UTI after *E. coli* [1,10]. This is probably because *Klebsiella* and *Proteus* produce potent urease which acts on urea to produce ammonia, rendering the urine alkaline [1]. *Enterobacter, Citrobacter, Serratia* and *Pseudomonas* species are more frequently found in hospital acquired UTI because their resistance to antibiotic favours their selection in hospital patients [1].

Seventeen positive cases were caused by gram-positive bacteria which represented 11.33% of all positive cases (Table-1). Gram-positive bacteria are known to be involved in the UTI. However the vast majority of UTIs are caused by gram-negative bacteria originating from the gut before entering the urethra[7]. Out of the 150 positive cases, 96(64%) were from female patients, while 54 (36%) of the cases were from males (Table-2). It is known that the incidence of UTI is generally higher in females than the males worldwide and for several reasons [1,7,10].

Antibiotic sensitivity: The standard disk diffusion method was used to determine the sensitivity of all gram-negative bacterial isolates to several antibiotics. Results are shown in (Table-3). It is obvious that a high percentage of all isolates were resistant to all used antibiotics except the nitrofurantion to which all tested isolates were sensitive. Nitrofurantion is an effective antibiotic in the treatment of UTI all over the world [2,23]. 75% of *E. coli* isolates (the leading causative agent) and 86% of all isolates were resistant to ampicillin (Table-3). The ampicillin is one of the common antibiotic used for the treatment of UTI [10,21,23], and the spread of resistance to this antibiotic in the Iraqi strain represent a major problem in treatment of the infection. It was reported in several parts of the world that the wide spread of ampicillin resistance is due to self transmissible plasmids carrying ampicillin resistance gene [22,24,25,26]. The second most effective antibiotic agent for all isolates was Nalidixic acid (25% of all isolates were resistant), while the Gentamycin was the third most effective antibiotic (39% were resistant) (Table-3). Both antibiotics are known to be effective in the treatment of UTI worldwide [2,27]. Generally speaking the percentages of resistance to antibiotics reported in this study are higher than those reported in some other part of the world [2,23,27]. This is a reflection for the misuse of antibiotics.

Many of the antibiotics resistance genes were found to be carried on self transmissible or mobilizable plasmids, and the transfer of such plasmids from one strain to another via conjugation was one of the major reasons for spreading the antibiotics resistance between bacterial population specially those belong to the family *Enterobacteriaceae* which represent the major causative agents for UTI [17,18,21,25,27].

Minimum Inhibitory Concentration: In order to determine the proper concentrations of antibiotics to be used in the selective media for selection of transconjugants, the minimum inhibitory concentration (MIC) for several multiresistant isolates (Donor) and *E.coli* HB101 (recipient) were determined. Results in (Table-4) showed that the standard strain *E.coli* HB101 was resistant to rifampicin (RA) and the MIC was 30µg/ml. However it was sensitive to all other antibiotics and the MICs were less than 10µg/ml,which was the lowest concentration used in the experiment. On the other hand, the three multiresistant *E. coli* donor strain were all more sensitive to rifampicin than *E. coli* HB101 but they were resistant to all other antibiotics tested in the experiment. Their MICs to all antibiotics were higher than that of *E.coli* HB101. According to the MIC values obtained it was decided to add rifampicine (30µg/ml) and tetracycline (30µg/ml) to the media for the selection of transconjugants resulted from conjugation between *E. coli* strain. The MIC value for other *Enterobacteriaceae* donor strains are shown in (Table-4) and accordingly (30µg/ml) tetracycline and rifampicine were used for the selection of transconjugants in all experiments.

Conjugation: Results of conjugation experiments between different multiresistant donor strains and *E. coli* HB101are shown in (Table-5). Conjugation mixture of three *E. coli* donor and *E. coli* HB101were plated on media containing rifampicin and tetracycline to select for tetracycline resistance transconjugants. Tetracycline resistant transconjugants were detected in all three cases and the conjugation frequencies were similar. It was concluded that tetracycline resistance marker in the three *E. coli* strains is a plasmid mediated maker.

Five tetracycline resistant transconjugants from each case were tasted for the transfer of other donor markers by rechecking their sensitivity to all antibiotics to which the donor strains are resistant. It was found that chloramaphenicol resistance, streptomycin resistance and trimethoprime + sulfamethoxazole resistance markers were all transferred with the tetracycline resistance in all tested transconjugants (Table-5). These results indicated that the tetracycline, chloramaphenicol, streptomycin and trimethoprime+Sulfamethoxazole resistance genes of the three *E. coli* strains are located on one selftransmissible plasmid. It seems that this plasmid is widely spread in the Iraqi isolates since it was detected in all the three *E. coli* strains. Studies in various parts of the world have demonstrated that different kinds of antibiotic markers including tetracycline resistance, streptomycin resistance, chloramphenicol resistance, trimethoprim + sulfamethexazole resistance are located (either singly or in combination of two or more) on plasmids of *E. coli* strains isolated from UTIs, and such plasmids plays an important role in spreading antibiotic resistance among the causative agents of UTI [16,17,21,22,24,26].

It was also reported that the wide spread of ampicillin resistance in bacterial strains causing UTIs is due to self transmissible plasmids carrying ampicillin resistance gene [22,24,25,26]. However the ampicillin resistance gene did not transfer by conjugation during this study (Table-5). This does not necessarily means that the ampicillin resistance gene is not located on a self transmissible plasmid in the studied strains because the selection for transconjugent was done on the bases of tetracycline resistance transconjugant and not for ampicillin resistance transconjugants. It is possible that the ampicillin resistance gene is located on conjugative plasmid other than the one carrying TE^r , C^r , S^r and SXT^r genes.

No conjugation was detected between *E. coli* and other member of the family *Enterobacteriaceae* (i.e. *Proteus, Serratia* and *Acinitobacter*) (Table-5). It is known that the conjugation process between bacterial strains belong to the same species is more common

than conjugation between members of different species. However several studies reported that the antibiotic resistance can be spread between different members of *Enterobacteriaceae* and related bacteria via conjugation [27]. The multiresistant conjugative plasmid detected in *E. coli* strain during this study deserve more detailed investigation because it seems to be widely distributed in the Iraqi *E. coli* strains isolated from UTIs.
Characterization of this plasmid and determination of its exact role in spreading the antibiotic resistance will be certainly important for the understanding of the wide spread of antibiotic resistance among bacterial strains causing UTI.

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Table No. (1): Percentage of bacterial species isolated from urinary tract infections

	Bacterial species	Number	Percentage (%)
1 -	Escherichia coli	60	40
2 -	Klebsiella pneumoniae	28	18.67
3 -	Proteus mirabilis	27	18.00
4 -	Enterobacter aerogenes	4	2.67
5 -	Citrobacter ferundii	4	2.67
6 -	Enterobacter cloacae	3	2.00
7 -	Acinitobacter calco var anitral	3	2.00
8 -	Pseudomonas maltophilia	2	1.33
9 -	Pseudomonas aeurogenosa	1	0.67
10-	Serratia marcescens	1	0.67
11-	Staphylococcus sp.	10	6.67
12-	Streptococcus sp.	7	4.66
	Total	150	100

Table No.(2): Prevalence of urinary tract infection in males and females.

Sex	Number of patients	Percentage (%)
Female	96	64
Male	54	36
Total	150	100

Table No.(3): Percentage of resistance to antibiotics by the bacterial strains isolated from urinary tract infection and the best three antibiotics

Isolates	Percentage of resistance to:												The best three antibiotics
	AM	C	N	RA	NA	S	TE	SXT	FT	GN	AMX	CX	
E. coli	75	50	70	73	17	65	77	88	0	53	75	20	FT, NA, CX
K. peneumoniae	89	54	57	100	32	50	43	86	0	61	100	32	FT, NA, CX
P. mirabilis	74	74	67	89	11	78	85	93	0	56	85	30	FT, NA, CX
Ent. aerogenes	100	75	100	100	0	50	75	100	0	75	100	75	FT, NA, S
C. ferundii	25	50	50	75	25	50	50	100	0	25	50	0	FT, NA, GM
Ent. Cloacae	100	33	100	100	33	67	33	67	0	0	100	100	FT, GN, NA
A. calco var anitrat	100	100	33	0	33	67	67	100	0	67	100	67	FT, RA, NA
Ps. maltophilia	100	100	50	50	0	0	100	100	0	50	50	50	FT, NA, S
Ps. aeroginosa	100	100	100	100	100	100	100	100	0	0	100	100	FT, GN
Ser. marcescens	100	0	0	0	0	100	100	100	0	0	100	0	FT, NA, GN
Total	86	64	63	69	25	63	73	84	0	39	86	48	

Table No.(4): Minimum inhibitory concentration for different multiresistant donor strains and the recipient strain *E. coli* HB101.

	Bacterial strain	Antibiotic MICs µg/ml							
		AM	RA	NA	S	TE	GM	AMX	CX
1 -	*E. coli* HB101	< 10	> 30	< 10	< 10	< 10	< 10	< 10	< 10
2 -	*E. coli* Z4	25	12.5	*-	25	30	30	-	30
3 -	*E. coli* Z 64	25	25	30	25	30	30	30	30
4 -	*E. coli* Z 76	30	25	30	25	30	30	35	30
5 -	*P. mirabilis* Z 88	25	20	< 10	35	30	> 40	-	30
6 -	*Ser. marcescena* Z 109	-	<20	20	20	30	25	30	20
7 -	*A. calco var anitrat* Z 45	-	< 20	35	25	35	20	-	-

* - = Not done

Table No.(5): Conjugation between multiresistant bacterial donor strains and the recipient *E. coli* HB101 strain.

	Donor and its resistance pattern	Recipient and its resistance pattern	Donor marker selected	Conjugation frequency	Other donor markers transferred
1	*E. coli* Z4 Am^r, C^r, NA^r, S^r, TE^r, SXT^r, GN^r, AMX^r, CX^r	*E. coli* HB101 RA^r	TE^r	1.5×10^{-4}	C^r, S^r, SXT^r
2	*E. coli* Z64 Am^r , C^r, N^r, NA^r, S^r, TE^r, SXT^r, GN^r, AMX^r	*E. coli* HB101 RA^r	TE^r	2×10^{-4}	C^r, S^r, SXT^r
3	*E. coli* Z76 Am^r, C^r, S^r, TE^r, SXT^r, GN^r, AMX^r	*E. coli* HB101 RA^r	TE^r	3×10^{-4}	C^r, S^r, SXT^r
4	*P. mirabilis* Z88 Am^r, C^r, N^r, TE^r, SXT^r, GN^r, AMX^r	*E. coli* HB101 RA^r	TE^r	* -	-
5	*Ser. marcescens* Z109 Am^r, C^r, S^r, TE^r, SXT^r, AMX^r	*E. coli* HB101 RA^r	TE^r	* -	-
6	*A. colco varanitrat* Z45 Am^r, C^r, $N.A^r$, TE^r, SXT^r, AMX^r, CX^r	*E. coli* HB101 RA^r	TE^r	* -	-

تشخيص سلالات البكتريا المعزولة من المرضى المصابين بالتهاب المجاري البولية ودور البلازميدات في مقاومتها للمضادات الحيوية

زينة غازي فيصل
فلاح عبد الجبار عطاوي
غالب حمزة البكري
قسم التقانة الأحيائية / كلية العلوم / جامعة النهرين

استلم البحث في :26 تموز 1999 قبل البحث في :26 تموز 1999

الخلاصة

عزلت مائة وخمسون سلالة بكتريا من المصابين بالتهاب المجاري البولية، تم تشخيص هذه السلالات فوجد أنها تعود لعشرة أنواع من البكتريا السالبة لصبغة جرام، وإلى جنسين من البكتريا الموجبة لصبغة جرام. وجد أن بكتريا *E. coli* هي المسبب الرئيس لالتهابات المجاري البولية حيث كانت تمثل 40% من جميع الحالات . وجاءت البكتريا *Klebsiella pneumonia, Proteus mirabilis* بالمرتبتين الثانية والثالثة وبنسب 18.67% و 18% على التوالي. أما أنواع البكتريا السالبة لصبغة جرام الأخرى المعزولة كانت تعود للأجناس *Enterobacter, Acinitobacter, Serratia, Citrobacter, Pseudomonas* . عشرة عزلات كانت تعود للجنس *Staphylococcus* وبنسبة 6.67% في حين عزلت سبعة سلالات تعود للجنس *Streptococcus* وبنسبة 4.66%. من مجموع 150 حالة كانت 96 (64%) منها معزولة من النساء في حين كانت 54 (36%) سلالة معزولة من الرجال.

أظهرت جميع العزلات نسبة مقاومة عالية لجميع المضادات الحيوية المستخدمة في الدراسة باستثناء النتروفيورانيشن، حيث كانت جميع السلالات المعزولة حساسة له. أما حامض الناليدكسيك فجاء ثانياً من حيث الفعالية (25% من السلالات مقاومة له) في حين جاء الجنتمايسين بالمرتبة الثالثة حيث كانت 39% من السلالات مقاومة له. كما أظهرت الدراسة أن 75% من سلالات *E. coli* و 86% من مجموع السلالات مقاومتها للامبيسيلين. أن النسب العالية للمقاومة للمضادات الحيوية هي إنعكاس لسوء استخدام المضادات الحيوية مما يشجع على زيادة نسب المقاومة في البكتريا.

أظهرت نتائج الاقتران أن سلالات *E. coli* تحتوي على بلازميد اقتراني يحمل الجينات المقاومة للتتراسايكلين والكلورامفينيكول والستربتومايسين والترايميثوبريم معاً ويبدو أن هذا البلازميد يتواجد بشكل واسع في السلالات المعزولة من العراق حيث أنه وجد في السلالات الثلاثة لبكتريا *E. coli* التي تمت دراستها. لم تحصل عملية الاقتران بين بكتريا *E. coli* والبكتريا العائدة للعائلة المعوية من الأجناس Acinitobacter ، Proteus و Serratia.

الكلمات المفتاحية: البكتريا، المقاومة للمضادات الحيوية، البلازميدات